Chen et al. (2003) similarly found expression of intestinal specific genes to be lower in EBV (+) stomach cancers, as compared with EBV (-) lesions. Regarding the regulation of MUC2 expression, Yamamoto et al. (2003) have demonstrated that Cdx2 interacts with the MUC2 promoter and activates MUC2 transcription. Lee et al. (2004) have previously shown that there is negative association between EBV infection and expression of MUC2 in stomach cancers, again in line with the our present data (Table 2). Therefore, we consider that the absence of Cdx2 and MUC2 is linked in EBV (+) stomach cancers.

We also here demonstrated that stomach cancers are more likely to be of N type in the EBV (+) group, in line with the previous report that EBV (+) stomach cancers have lower MUC5AC and MUC2 expression than their EBV (-) counterparts (Lee et al., 2004). EBV associated stomach carcinomas are reported to lack intestinal phenotypic expression (Chen et al., 2003) and most EBV (+) stomach cancers were here classified phenotypically as N or G types (Table 3). Nakamura et al. (2005) also previously showed the G type to be more common in EBV (+) cases.

Several reports have shown that EBV (+) stomach

**Table 6.** Comparison of phenotypic markers in differentiated and undifferentiated regions in EBV (+) and EBV (-) stomach cancer cases.

| Case No. | EBER-ISH | Histology | Phenotypes in total area | Phenotypical marker expression in each region | | Ratio of N types in U region[a] |
|---|---|---|---|---|---|---|
| | | | | D region | U region | |
| 1 | + | D>U | G | G | N | N=3/6 (50%) |
| 2 | + | D>U | I | I | I | |
| 3 | + | U>D | G | G | G | |
| 4 | + | U>D | G | G | G | |
| 5 | + | U>D | G | G | N | |
| 6 | + | U>D | I | I | N | |
| 1 | – | D>U | GI | GI | GI | N=0/9 (0%) |
| 2 | – | D>U | I | I | I | |
| 3 | – | U>D | G | G | G | |
| 4 | – | U>D | G | G | G | |
| 5 | – | U>D | G | G | G | |
| 6 | – | U>D | GI | GI | GI | |
| 7 | – | U>D | GI | GI | I | |
| 8 | – | U>D | GI | GI | I | |
| 9 | – | U>D | I | I | I | |

[a]: P<0.02 (Fisher's exact test). Abbr.: D, differentiated; U, undifferentiated; G, gastric; I, intestinal; GI, gastric-and-intestinal-mixed; N, null.

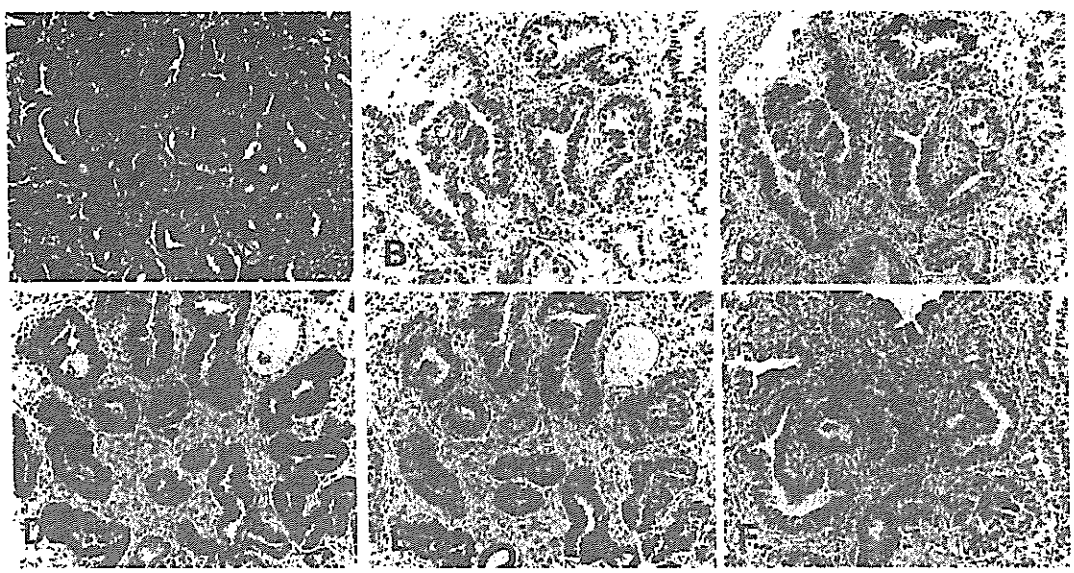


Fig. 2. An EBV (-) stomach cancer. **A.** HE staining. **B.** Cdx2 nuclear staining is positive in some cancer cells. **C.** MUC2 expression is detected in the cytoplasm of some tumor cells. **D.** MUC5AC is present in the cytoplasm of the cancer cells. **E** MUC6 is apparent in the cytoplasm of some tumor cells. **F.** EBER-1 is negative in the nuclei of the cancer cells. x 200; EBER-1, EBV-encoded small RNA-1.

cancers are most often undifferentiated histopathologically, according to the Japanese Classification of Gastric Carcinomas (Yanai et al., 1997; Wu et al., 2000; Lee et al., 2004). EBV (+) stomach cancers are more frequently moderately differentiated tubular adenocarcinomas (tub2), and solid poorly differentiated adenocarcinomas (por1) as compared with other histological types (Carrascal et al., 2003). To avoid bias, phenotypic expression was here evaluated in morphologically matched samples for EBV (+) and EBV (-) cases.

Regarding the histogenesis of EBV associated stomach cancers, Fukayama et al. (2001) previously suggested the hypothesis that they develop by clonal expansion of rare EBV-infected epithelial cells within stomach mucosa. EBV infection of intestinal metaplastic cells is unlikely (Fukayama et al., 2001). We have argued that the origin of stomach cancers is from progenitor cells specializing towards mucous differentiation in the fundic/pyloric glands, rather than intestinal metaplastic glands (Tatematsu et al., 2005). With EBV infection the histogenesis may be from cells that are specialized towards mucous differentiation in the fundic/pyloric glands, harboring neither typical gastric nor intestinal phenotypic expression.

In the present study, inflammatory response in the surrounding non-neoplastic mucosa was not statistically different between EBV (+) and EBV (-) cases. So EBV may not have significantly induced inflammatory cell infiltration in our Columbia cases. The Cdx2 expression in the intestinal metaplastic glands was also lower in non-neoplastic mucosa of EBV (+) cases, despite no EBV infection being observed by in situ hybridization. However, the presence of EBV in non-carcinomatous surrounding mucosa of EBV (+) stomach cancers has been detected by immunostaining of EBNA-1 and latent membrane protein 1 (LMP-1) (Yanai et al., 1997a,b). Hayashi et al. (1996) detected EBV in gastric glands with IM. Yanai et al. (1999) reported the evidence that all eight lesions of EBER-1-positive gastric carcinomas had intestinal metaplasia in the background among 8 EBER-1-positive stomach carcinomas. In contrast, Kaizaki et al. (1999) reported that only 13% of EBV (+) stomach cancers were surrounded by intestinal metaplasia, in contrast to 41% of EBV (-) ones. Zur Hausen et al. (2004) concluded that EBER-1/2 transcripts were restricted to the carcinoma cells in accordance with exclusive positivity of EBNA-1 immunohistochemistry (IHC) to the tumor cells. Negative LMP-1 IHC in all cases tested and absence of EBER-1/2 transcripts in preneoplastic gastric lesions (intestinal metaplasia and dysplasia) strongly suggested that EBV could only infect neoplastic gastric cells, indicating it as a late event in gastric carcinogenesis.

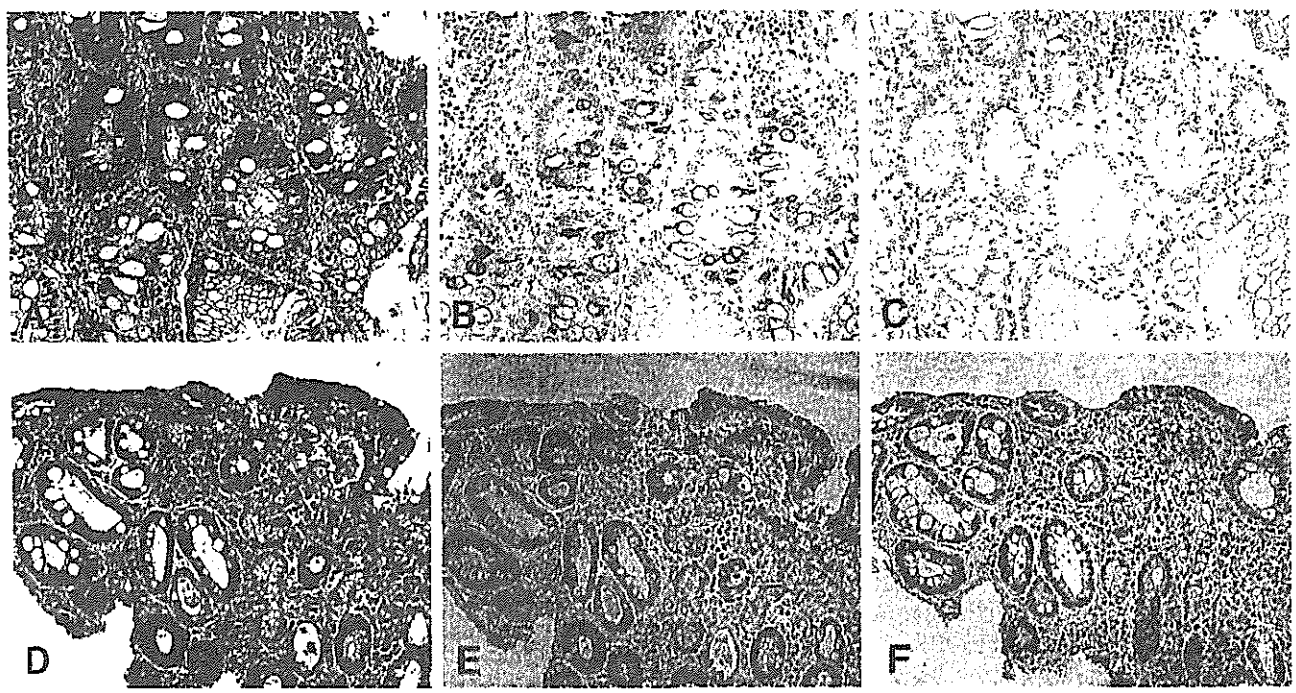


Fig. 3. Expression of MUC2 and Cdx2 in intestinal metaplastic glands in tissue surrounding adenocarcinomas. EBV (+) **(A-C)** and EBV (-) **(D-F)** stomach cancers. **A and D.** HE staining. **B and E.** MUC2 is detectable in the cytoplasm of intestinal metaplastic glands. **C and F.** No Cdx2 nuclear staining in intestinal metaplastic glands in an EBV (+) case (C) in contrast to apparent nuclear staining in an EBV (-) case. x 200.

Thus down regulation of Cdx2 might not be due to infection of EBV to the surrounding mucosa. EBV (+) stomach cancer and surrounding intestinal metaplasia were similar to down regulation of Cdx2. We considered EBV might have infected the progenitor cell or stem cell after late event in gastric carcinogenesis and intestinal metaplasia, and the down regulation of Cdx2 were similar mechanism to EBV (+) stomach cancer and surrounding intestinal metaplasia. Further studies of EBV infection in non-neoplastic stomach epithelia appear warranted.

In conclusion, EBV (+) stomach cancers are characterized by a relative lack of intestinal phenotypic expression, including Cdx2, and only occasional presence of gastric phenotypic expression. The progenitor cell may thus be specialized towards mucous differentiation in the fundic/pyloric glands.

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# Significance of Lymphatic Invasion on Regional Lymph Node Metastasis in Early Gastric Cancer Using LYVE-1 Immunohistochemical Analysis

*Ai Fujimoto, MD,[1,7*] Yukio Ishikawa, MD,[1*] Yuri Akishima-Fukasawa, MD,[1] Kinji Ito, MD,[1] Yoshikiyo Akasaka, MD,[1] Seiichi Tamai, MD,[2] Tadaaki Maehara, MD,[3] Hideko Kiguchi, MD,[4] Kentaro Ogata, MD,[5] Chiaki Nishimura, PhD,[6] Kazumasa Miki, MD,[7] and Toshiharu Ishii, MD[1]*

**Key Words:** Early gastric cancer; Lymph node metastasis; Lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) antibody; Lymphatic invasion

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## Abstract

*It has been reported that lymphatic invasion is a predictor for lymph node metastasis in early gastric cancer (EGC); however, it has been impossible to differentiate between lymphatic invasion and blood vessel invasion using current staining techniques. We studied the significance of lymphatic invasion on regional lymph node metastasis in EGC by using human lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) antibody, specific to lymphatic vessels, and von Willebrand factor (vWF) antibody, specific to the blood vessels, to clearly distinguish these vascular tissues.*

*EGC tissues were obtained from 66 node-positive and 66 node-negative subjects and were matched by age and sex. These tissues were immunostained with antibodies against LYVE-1 and vWF. Multivariate logistic regression analysis demonstrated that lymphatic invasion was a significant independent predictor for regional lymph node metastasis (odds ratio, 4.667;* P = *.0094), whereas blood vessel invasion was not. Thus, lymphatic invasion identified by LYVE-1 antibody could predict the existence of regional lymph node metastasis in EGC.*

The postsurgical 5-year survival rate for patients with early gastric cancer (EGC) is more than 90%[1-4]; however, in approximately 10.2% to 14.4% of EGC cases, cancer metastasizes to the regional lymph nodes, causing patient death due to subsequent systemic spread.[2,5-10] Lymph node metastasis has emerged as a significant independent indicator of poor long-term survival in EGC.[7,11]

Recent availability of endoscopic techniques and laparoscopic resection for patients with EGC has improved the quality of life by minimizing invasive procedures.[2,7,12-16] However, there is no method to precisely predict the existence of lymph node metastasis without using invasive procedures, thus limiting the use of minimally invasive techniques.

Among the routes by which EGC can metastasize to regional lymph nodes, metastasis through the lymphatics at the primary site is a major candidate. Although there are many small lymphatics and blood capillaries present at the primary site in gastric cancer, it is difficult to distinguish between these 2 vessels by using H&E staining.[17] However, we recently developed a polyclonal antibody against human lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1; to be released by DakoCytomation, Glostrup, Denmark, in 2007), an immunohistochemical marker that enables us to recognize lymphatics.[18] Thus, it has become possible to distinguish objectively between the 2 types of vascular tissues by immunohistochemical analysis for LYVE-1 and von Willebrand factor (vWF).

In this study, we attempted to examine the significance of lymphatic invasion as a predictor for regional lymph node metastasis by performing immunohistochemical techniques using the LYVE-1 antibody.

## Materials and Methods

The materials were surgical samples of stomach and regional lymph nodes obtained from 66 patients who had undergone curative resection for gastric cancer at Toho University Hospital, Tokyo; the National Defense Medical College Hospital, Tokorozawa; Saiseikai Kanagawa-Ken Hospital, Yokohama; Hiratsuka Municipal Hospital, Hiratsuka; and Ida Municipal Hospital of Kawasaki City, Kawasaki, Japan, between January 1989 and December 2004. Histopathologic examination of all 66 node-positive specimens confirmed that although the cancer had invaded only the mucosa and submucosal layer of the stomach, it had metastasized to the regional lymph nodes. Among the cases with lymph node metastases, only 12 (18%) had intramucosal carcinomatous invasion and 54 (82%) had invasion extending to the submucosal layer.

Of the 66 patients, 51 underwent partial or subtotal gastrectomy and 15 underwent total gastrectomy. Curative surgery was defined as the removal of all gross cancers and the demonstration of tumor-negative surgical margins by microscopic examination of the total circumference. The patients were free of other types or degrees of invasion, distant visceral metastases, and complications due to other visceral cancers. The patients were not given chemotherapy preoperatively. Stomach specimens from 594 patients with EGC but without regional lymph node metastasis were extracted from the archives and used to identify age- and sex-matched control cases for each of the node-positive patients; 66 such control cases were finally sorted at random and used in this experiment. Of the 66 cases without lymph node metastases, 40 (61%) had invasion restricted to intramucosal carcinomatous invasion and 26 (39%) had invasion extending to the submucosal layer. Written informed consent to use the tissue samples was obtained from all patients.

The surgically resected stomachs were generally opened along the greater curvature, pinned on a cork board, and fixed in 10% formalin. After careful gross inspection and photography, each tumor was cut into 4-mm slices parallel to the major axis of the specimen and also cut parallel to the minor axis at the half-way mark of the major axis. If the tumors were smaller than 30 mm, all slices from the tumor were used; however, if the tumors were larger than 30 mm, only the slices obtained from the center of the tumor were used. The first cut was made in the middle of the tumor, followed by cuts above and below the middle mark to obtain the necessary slices. First, 1 horizontal row across all blocks and 2 rows each above and below the first row were sliced thin. The slices were embedded in paraffin, cut into 3-μm-thick sections, and treated by double staining with Victoria blue and H&E dyes to aid the identification of blood vessel structures, especially with regard to veins.

### Immunohistochemical Analysis

Immunohistochemical staining with the LYVE-1 antibody, previously raised against a LYVE-1 polypeptide fragment,[19] was carried out after dewaxing and dehydration of the thin-sectioned specimens. The sections were pretreated with 10 mmol/L of citrate buffer solution (pH 6.0) for 15 minutes at 95°C and then with 40 μg/mL of Proteinase K (DAKO, Carpinteria, CA) for 3 minutes at room temperature. After washing in distilled water, the sections were incubated with LYVE-1 antibody (diluted 1:200) for 1.5 hours at room temperature, washed in tris(hydroxymethyl)aminomethane (Tris)-buffered saline (TBS) containing polysorbate 20, and treated with the Catalyzed Signal Amplification II kit (DAKO) according to the manufacturer's instructions. The immunostaining was visualized with diaminobenzidine tetrahydrochloride, followed by counterstaining with hematoxylin.

For vWF immunohistochemical staining, the sections were dewaxed, dehydrated, and pretreated with 10 mmol/L of citrate buffer solution (pH 6.0) for 15 minutes at 95°C. After washing in TBS, they were treated with 3% hydrogen peroxide for 10 minutes and then with 3% nonfat dried milk in TBS containing polysorbate 20 for 30 minutes. The sections were then incubated with antihuman vWF antibody (diluted 1:25; DAKO) for 2 hours at room temperature. A further wash in TBS was followed by treatment with a peroxidase-labeled polymer conjugated to goat and antirabbit or antimouse immunoglobulins (EnVision+ kit, DAKO) for 30 minutes at room temperature. The immunostaining was visualized with diaminobenzidine tetrahydrochloride, followed by counterstaining with hematoxylin.

### Histopathologic Variables

We assessed the relationships between the following histopathologic variables: location, size, grade of differentiation, cancerous ulceration, and lymphatic and blood vessel invasion. For size of the cancer, the major axis of the primary EGC lesion was measured. For grade of differentiation, the histopathologic type at the primary site was categorized as papillary adenocarcinoma, well-differentiated adenocarcinoma, moderately differentiated adenocarcinoma, poorly differentiated adenocarcinoma, and signet-ring cell carcinoma according to the World Health Organization classification with Japanese modification.[19] For statistical treatments, we identified the first 3 types of differentiation as a low-grade malignancy group and the latter 2 types as a high-grade malignancy group according to the conventionally accepted relationship between the type of cancer and biologic behavior based on histopathologic classification.

For this analysis, vascular invasion was defined as invasion and adherence of cancer cells to the inside cell walls of the lymphatic or blood vessels. Lymphatic and blood vessel invasion was considered to be present when we could observe




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at least 1 vessel invaded by cancer cells. We examined the location of lymphatic and blood vessel invasion by imaging an entire primary tumor. After immunostaining for endothelial cells of lymphatic and blood vessels, double staining with Victoria blue and H&E dyes was also used to aid the identification of elastic fibers in the vein.

For all histopathologic variables, each macroscopic record and microscopic slide was analyzed by pathologists (A.F., Y.I., and T.I.) to reach consensus.

### Statistical Methods

Statistical analyses were performed using the $\chi^2$ test, Fisher exact test, and the Mann-Whitney $U$ test to assess the significance of the impact of each subset of histopathologic variables on lymph node condition. Univariate and multivariate logistic regression analyses were carried out to identify independent predictive factors for lymph node metastasis. Differences at a $P$ value of less than .05 were considered statistically significant. The StatView program (SAS Institute, Raleigh, NC) was used for all analyses.

**Table 1**
**Comparison of Histopathologic Parameters by Lymph Node Status in Early Gastric Cancer**

| Variables | Node Status: Negative (n = 66) | Node Status: Positive (n = 66) | P |
|---|---|---|---|
| Location of primary cancer in gastric surface area | | | NS* |
| Upper third | 10 (15) | 13 (20) | |
| Middle third | 41 (62) | 29 (44) | |
| Lower third | 15 (23) | 24 (36) | |
| Mean size (mm) | 26.9 | 37.1 | .0005† |
| Cancerous ulceration | 13 (20) | 23 (35) | NS‡ |
| Grade of cancer differentiation | | | .0235‡ |
| Low-grade malignancy | 43 (65) | 33 (50) | |
| High-grade malignancy | 23 (35) | 33 (50) | |
| Lymphatic invasion | 6 (9) | 21 (32) | .0021‡ |
| Blood vessel invasion | 4 (6) | 13 (20) | .0352‡ |

NS, not significant.
* $\chi^2$ test.
† Mann-Whitney $U$ test.
‡ Fisher exact test.

## Results

### Comparison of Variables Between Node-Negative and Node-Positive Groups

Differences in histopathologic variables between node-negative and node-positive groups are given in Table 1. For tumor size, the Mann-Whitney $U$ test revealed a significant difference between the groups ($P$ = .0005). There was a significant difference in cancer differentiation between the groups as assessed by the Fisher exact test ($P$ = .0235). Furthermore, the node-negative group had differentiation predominantly in the low-grade malignancy range compared with the node-positive group.

The 2 varieties of vessels were easily distinguished by LYVE-1 Image 1A and vWF Image 1B immunostaining. The frequencies of lymphatic and blood vessel invasion were both significantly greater in the node-positive EGCs than in

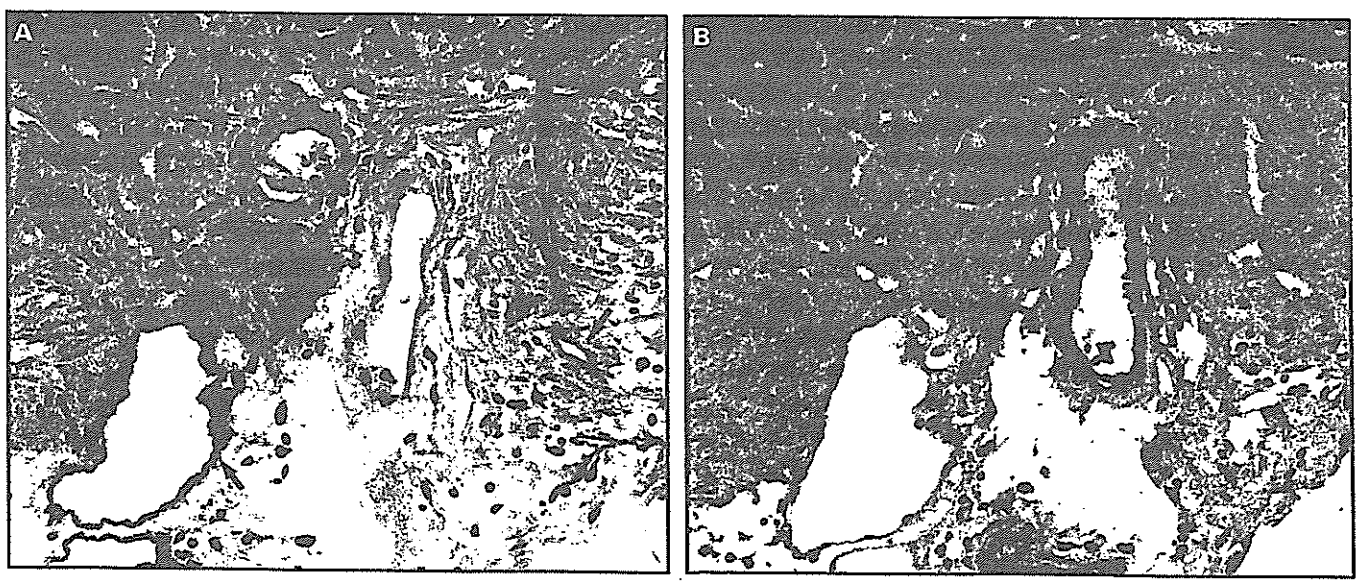


**Image 1** Discrimination of lymphatic vessels from blood vessels at the same site of a normal mucosa. **A**, Lymphatic vessel is lined with lymphatic vessel endothelial hyaluronan receptor-1–positive endothelial cells just beneath the lamina muscularis mucosae (arrows) (×400). **B**, Blood vessel is lined with von Willebrand factor–positive endothelial cells at the same site as in **A**. There is a blood vessel containing blood components within its lumen (arrows) (×400).


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the node-negative EGCs ($P = .0021$ and $P = .0352$, respectively; Fisher exact test). On the other hand, there was no significant difference in the frequency of lymph node metastasis for location of cancer in the stomach and cancerous ulceration.

### Univariate and Multivariate Logistic Regression Analyses of Histopathologic Variables as Predictors of Lymph Node Metastasis

The size of cancer, a low grade of cancer differentiation, lymphatic invasion, and blood vessel invasion were significant independent predictors for lymph node metastasis by univariate logistic regression analysis ▮Table 2▮. When multivariate analysis was undertaken on these 4 factors, significant predictors were size of primary cancer and lymphatic invasion ▮Table 3▮.

### Observation of Lymphatic and Blood Vessel Invasion

Of the 66 cases with lymph node metastases, 21 had lymphatic invasion and 13 had blood vessel invasion. Of the 66 cases without lymph node metastases, 6 had lymphatic invasion and 4 had blood vessel invasion. In 9 cases, lymphatic and blood vessel invasion were present.

We studied the spatial distribution of lymphatic and blood vessel invasion in relation to structure of the gastric wall of the primary cancer for the 132 node-positive and node-negative EGCs. Lymphatic invasion was mainly recognized just beneath the lamina muscularis mucosae (15/27 [56%]) ▮Image 2A▮ and ▮Image 2B▮ and in the submucosa (only in submucosa, 8/27 [30%]; in lamina muscularis mucosae and submucosa, 3/27 [11%]) and rarely occurred only within the mucosa (1/27 [4%]). When lymphatic invasion was assessed in terms of the portion of the primary cancer, it was frequently seen at the cancer periphery (17/27 [63%]) and in the central and peripheral portions (6/27 [22%]) of the cancer. Lymphatic invasion alone was rare in the central part of the cancer (4/27 [15%]).

Blood vessel invasion was mostly recognized in large blood vessels that exhibited a venous structure, which exists within the submucosa (12/17 [71%]) ▮Image 3A▮. When blood vessel invasion was assessed in relation to the portion of the primary cancer, invasion was seen in the central and peripheral portions (4/17 [24%]), in central portions (7/17 [41%]), and at the periphery (6/17 [35%]) of the primary cancer at almost equal frequency. vWF staining ▮Image 3B▮ was used to identify only 1 case of invasion by cancer cells in the blood vessel, whereas the other 16 cases were analyzed by double staining with Victoria blue and H&E dyes.

## Discussion

The present study is the first in which the significance of lymphatic invasion identification on regional lymph node metastasis in EGC was investigated by using immunohistochemical markers for lymphatic and blood vessels simultaneously. Immunostaining using LYVE-1 antibody enabled us to objectively distinguish the presence of lymphatic and blood vessels at the site that are apt to be overlooked as vacant interstitial spaces when using only H&E staining. In fact, by using this new antibody, we could observe considerably more lymphatics invaded by cancer cells compared with conventional staining methods.

The size of the primary tumor,[5,8,9,20,21] undifferentiated histopathologic features,[5,8,10,22] lymphatic or blood vessel invasion,[2,5,9,10,20,21,23,24] and cancerous ulceration[2,12,13] have been

▮Table 2▮
**Univariate Logistic Regression Analysis of Node-Positive Early Gastric Cancers**

| Parameter | Odds Ratio | 95% Confidence Interval | *P* |
|---|---|---|---|
| Location (middle and upper vs lower)* | 1.990 | 0.926-4.277 | NS |
| Size | 1.028 | 1.008-1.047 | .0046 |
| Cancerous ulceration | 2.181 | 0.989-4.806 | NS |
| Grade of cancer differentiation (low vs high) | 2.601 | 1.157-5.846 | .0207 |
| Lymphatic invasion | 4.667 | 1.740-12.512 | .0022 |
| Blood vessel invasion | 3.802 | 1.169-12.363 | .0264 |

* Middle, upper, and lower thirds of the gastric surface area.

▮Table 3▮
**Multivariate Logistic Regression Analysis of Node-Positive Early Gastric Cancers**

| Parameter | Odds Ratio | 95% Confidence Interval | *P* |
|---|---|---|---|
| Size | 1.030 | 1.010-1.049 | .0023 |
| Grade of differentiation (low vs high) | 2.073 | 0.952-4.517 | NS |
| Lymphatic invasion | 3.987 | 1.404-11.325 | .0094 |
| Blood vessel invasion | 3.646 | 0.996-13.353 | NS |




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considered as parameters of lymph node metastasis in EGC. In this study, the multivariate logistic regression analysis demonstrated that lymphatic invasion was a significant independent predictor of regional lymph node metastasis in EGC, whereas blood vessel invasion was not a significant predictor.

It is desirable that logistic analyses be conducted on a large sample and the results analyzed blindly, but it was not possible in the present study because EGC with lymph node metastases accounts for only 10% of reported cases in literature and although our data in the past 15 years on node-negative cases are vast, pathologic materials and information on patients' backgrounds were insufficient. Thus, subjects in 2 groups in the present study were matched by age and sex. Although we tried to avoid biases on comparative variables as much as possible, it is undeniable that unseen variables could have influenced our results. The results of the present study were also confirmed by results obtained in previous studies.

For lymphatic invasion, because the lymphatics are a route to lymph nodes, it would be reasonable to conclude that lymphatic invasion is one of the predictors of lymph node metastasis. However, previous studies have not been able to clarify the differences between lymphatic and blood vessel invasion.

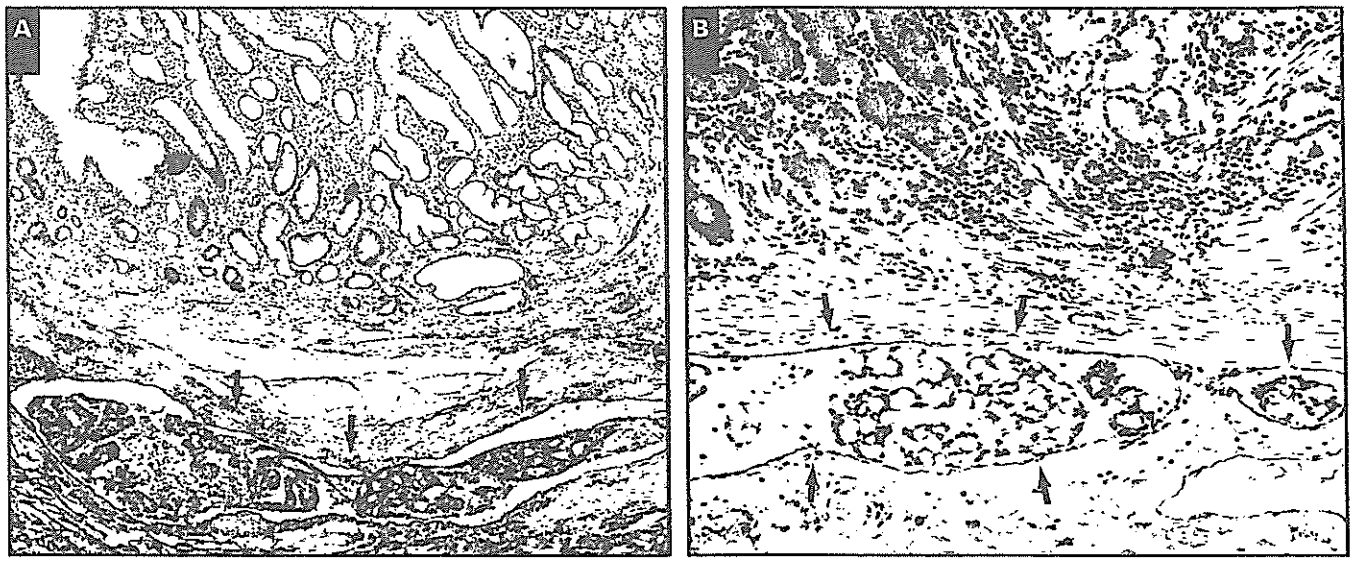


**Image 2** The arrows in the image indicate the circumferences of the lymphatic vessels. **A** and **B**, Lymphatic invasion is evident just beneath the lamina muscularis mucosae (**A**, LYVE, ×100; **B**, LYVE, ×200). LYVE, lymphatic vessel endothelial hyaluronan receptor-1.

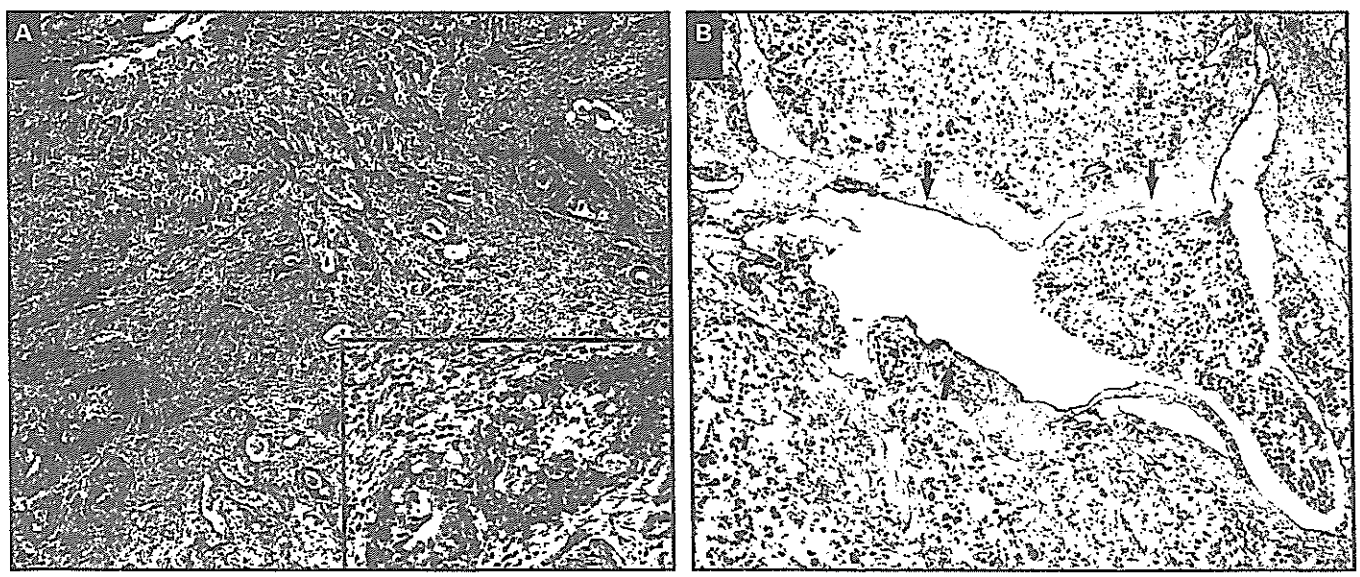


**Image 3** **A**, Venous invasion as recognized by double staining with H&E and Victoria blue dyes (arrow) (×100). Inset, High-power view of the same vein (×400). **B**, Blood capillary invasion as recognized by von Willebrand factor immunostaining. The arrows indicate the circumference of the blood capillary (×150).


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In 1999, Banerji et al[25] identified the human LYVE-1 molecule as a major receptor of hyaluronan at the surface of the lymphatic endothelium. However, immunohistochemical detection using a previous LYVE-1 antibody was possible in only some pathologic tissue samples but difficult in most pathologic tissue samples.

We developed a new LYVE-1 antibody. Our LYVE-1 antibody has a higher specificity for lymphatic endothelial cells than previous LYVE-1,[25] podoplanin,[26] prox-1,[27] desmoplakin,[28] D6,[29] the mannose receptor,[30] and D2-40.[31] Furthermore, our LYVE-1 antibody could detect lymphatics under various conditions[18] that previously caused difficulty in detection.

By using these methods, we observed lymphatic invasion in EGC. Most lymphatics were found just beneath the lamina muscularis mucosae, and they were generally not seen within the mucosa. It has been accepted that the lymphatics arise only in the mucosa below the bases of gastric glands. Lymphatic vessels in the mucosal layer have no endothelial lining and so could be termed tissue channels rather than lymphatics.[32] Thus, because of these anatomic features, it is likely that lymphatics are not demonstrable in the mucosa by LYVE-1 even when a large number of such tissue channels are invaded by cancer cells in the gastric mucosa.

In addition, lymphatics were almost never found in the central portion of the primary cancer. However, lymphatics distributed at the periphery of the cancer, which remained intact, showed distinct LYVE-1 positivity. It is conceivable that the cancer cells make contact with lymphatics in the central portion of the primary tumor and destroy the lymphatic structure in that area. Microscopic examination of lymph vessel detected numerous lymph vessels, particularly those inside cancer masses with structural defects and fragmentation due to cancer cell invasion. The fragments from lymphangial epithelia were successfully stained with LYVE-1 antibody, suggesting the presence of lymphatic invasion. However, for the purpose of accuracy of the study, only cases with lymph vessels with complete structure were counted to confirm lymphatic invasion. Therefore, it is possible that because of the limitations of the antibody and statistical methods, only 30% are seen to have lymphatic invasion.

In recent years, minimal surgical procedures such as endoscopic techniques and laparoscopic resection have been developed to treat EGC; however, there remains a serious problem of ignoring the status of regional lymph nodes in such treatments. When we encounter lymphatic invasion at the primary site by microscopic examination of EGC, we need to consider the possibility of regional lymph node metastasis. Thus, the LYVE-1 antibody could be further explored by using it to identify lymphatic invasion in clinical situations by immunohistochemical analysis.

*From the Departments of [1]Pathology and [6]Medical Informatics, School of Medicine, Toho University, Tokyo; [7]Gastroenterology and Hepatology, Ohmori Medical Center, Toho University, Tokyo; [2]Pathology and [3]Surgery, National Defense Medical College, Tokorozawa; [4]Pathology, Saiseikai Kanagawa-Ken Hospital, Yokohama; and [5]Pathology, Ida Municipal Hospital of Kawasaki City, Kawasaki, Japan.*

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*Address reprint requests to Dr Fujimoto: Dept of Pathology, School of Medicine, Toho University, 5-21-16 Ohmori-Nishi, Ohta-Ku, Tokyo 143-8540, Japan.*

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*Review article*

# Gastric cancer screening using the serum pepsinogen test method

KAZUMASA MIKI

Division of Gastroenterology and Hepatology, Department of Internal Medicine (Ohmori), School of Medicine, Faculty of Medicine, Toho University, 6-11-1 Ohmori Nishi, Ohta-ku, Tokyo, 143-8541 Japan

**Abstract**

**The current status of gastric cancer screening, worldwide, as well as in Japan, using the serum pepsinogen test method, was reviewed. We performed a metaanalysis of sensitivity and specificity results from 42 individual studies (27 population-based screening studies: $n = 296\,553$ and 15 selected groups: $n = 4\,385$). Pooled pairs of sensitivity and false-positive rates (FPr) for pepsinogen I level ≤ 70 ng/ml; pepsinogen I/II ratio ≤ 3, had a sensitivity of 77%/FPr27%. The positive predictive value varied between 0.77% and 1.25%, and the negative predictive value varied between 99.08% and 99.90%. Therefore, we concluded that the definition of the pepsinogen test should include the pepsinogen I/II ratio, as consistency was obtained for both the population-based studies and the selected groups for those studies that used pepsinogen I serum levels together with the pepsinogen I/II ratio for screening for gastric cancer in high-incidence regions other than Japan. Individuals testing positive for extensive atrophic gastritis by serum pepsinogen levels undergo endoscopic examination to test for the presence of gastric cancer. We should increase the efficacy and cost-effectiveness of the gastric cancer screening system, by the identification of groups, at low-risk, as well as those at high-risk, of developing gastric cancer, using a combination of assays of serum *Helicobacter pylori* antibody titers and the concentration of pepsinogen I and II. In conclusion, the pepsinogen test method can be used as a screening test for high-risk subjects, rather than as a tool for screening for cancer itself. I hope that this pepsinogen test method will become a world standard for gastric cancer prevention in the near future, in other countries, as well as in Japan.**

**Key words** Gastric cancer · Cancer screening · High-risk group · Serum pepsinogen · *Helicobacter pylori* antibody

*Offprint requests to:* K. Miki
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## Introduction

Gastric cancer remains one of the leading causes of cancer-related death worldwide [1]. The highest rates of gastric cancer are in Costa Rica, Chile, and Japan; one of the lowest rates is in the United States. In Japan, a gastric cancer screening program was introduced in the 1960s as a public health service and this has gradually been extended to include the whole nation. Currently, screening is performed throughout the country, and more than 6 million people annually undergo screening provided by either a community service or in the workplace. As a result, thousands of stomach cancer cases are detected each year, and the cancer screening has greatly contributed to a reduction in gastric cancer mortality rates [2,3]. Screening most frequently includes the use of double-contrast barium X-rays or panendoscopy, as well as photofluorography. Recently, serum pepsinogen tests were introduced for mass screening to identify individuals at high risk for gastric cancer. Individuals testing positive for extensive atrophic gastritis, based on their serum pepsinogen levels, undergo endoscopic examination to test for the presence of gastric cancer. The results of the serum pepsinogen screening tests are comparable and, in some respects superior to, those of traditional screening [4–7]. The objective of this review is to describe the current status of gastric cancer screening, using the serum pepsinogen test method, worldwide, as well as in Japan.

Gastric cancer, despite a recent decline in its incidence, is still the second leading cause of cancer deaths in Japan. For this reason, much effort has been directed to the early detection of cancer, using mass screening programs throughout the country. At present, as described above, about 6 million people are screened annually, by photofluorography; however, the sensitivity of this method is by no means high if endoscopy is used as a yardstick [4,7,8]. In contrast, the measurement of

serum pepsinogens has recently gained attention as a new screening test for gastric cancer [4–12]. This method is particularly attractive given its lower cost and simplicity to administer relative to photofluorography [4,7–9,12,13].

## Serum pepsinogen

Serum pepsinogen consists of two biochemically and immunologically distinct types, namely, pepsinogen I (PGI) and pepsinogen II (PGII) (PGI is also called PG"A", and PGII is also called PG"C"). PGI is produced by chief and mucous neck cells in the fundic glands, while PGII is produced by these cells and also by cells in the pyloric glands and Brunner's glands [1,14–16]. It is widely accepted that serum pepsinogen levels reflect the functional and morphologic status of the gastric mucosa. As the fundic gland mucosa is reduced, PGI levels gradually decrease, whereas PGII levels remain fairly constant [4,6]. As a result, a stepwise reduction of the PGI/II ratio is closely correlated with the progression from normal gastric mucosa to extensive atrophic gastritis; this ratio of more than 3 has a sensitivity of 93.3% and specificity of 87.7% for the diagnosis of normal fundic gland mucosa [4,6].

## Pepsinogen test method

It is generally accepted that serum pepsinogen concentrations are related to gastritis, and gastric mucosal lesions, with a particular relationship to chronic atrophic gastritis (CAG) [4,5,17–19]. At least for intestinal-type [20] gastric carcinoma, CAG is considered to be a preceding condition in the sequential histopathological changes that lead to cancer [21–23]. Pepsinogen has therefore been used as a serological biopsy for more than 20 years in different countries and different sets of patients [24–33].

Generalized screening as it is practiced in Japan may not be feasible in other countries. Owing to its low positive predictive value, some authors [34] have reported their concern about the effectiveness and applicability of the pepsinogen test for gastric cancer screening in countries with a lower prevalence of gastric cancer than that in Japan. Furthermore, significant differences in methodologies may prejudice the assessment of consistency. For instance, different cutoff values are used for the definition of positivity; either pepsinogen I levels [28,35–37] or both pepsinogen I and II [14–16] are considered; and not all studies have considered other factors, such as sex, age, and smoking and drinking habits, and *Helicobacter pylori* infection, factors which are said to influence pepsinogen levels. Nevertheless, as a noninvasive test, pepsinogen screening deserves further evaluation.

Based on the assessment of consistency of diagnostic validity among studies, we first aimed to evaluate the use of pepsinogen as a screening for gastric cancer in terms of the best methodology (pepsinogen I alone, or pepsinogen I and II), and with regard to the best cutoff point. We also aimed to define the usefulness of pepsinogen tests for identifying individuals with CAG and other associated lesions; namely, intestinal metaplasia and low-grade dysplasia, as in most Western countries the strategies for an early diagnosis of gastric cancer have been focusing on follow-up protocols for these individuals. It is generally accepted that following up these lesions is required for an early diagnosis of gastric cancer [38].

## Review of the literature: pepsinogen test for gastric cancer screening

We performed a metaanalysis of sensitivity and specificity results from individual reports on the use of pepsinogen tests. An intrinsic cutoff effect was assumed, and a random-effect model was used for pooling, as reported previously [39]. After defining the search strategy (see "inclusion criteria" below), published articles on pepsinogen test validity were found, using a computer-aided search in the Medline database (PubMed) and data reports from Japan.

Articles in any language were considered. Quality criteria for the inclusion of a study were defined as follows:

1. Clear definition of the study population and of available data on variables such as age, sex, smoking or alcohol habits, and *Helicobacter pylori* infection had to be noted.
2. Only those studies in which gastric endoscopic examination (with biopsies) was performed as a reference test or gold standard were considered. Two different results were considered: diagnosis of gastric cancer; and diagnosis of lesions associated with gastric cancer, such as atrophy or dysplasia. It was assumed that, as diagnosis is based on histology, definitions had not changed since the time during which the studies took place, and it was also assumed that there were no differences between definitions used by Japanese and Western pathologists. Also, "adenoma" was considered to be a synonym of low-grade dysplasia. Of note, a discussion of histopathological classifications is beyond the scope of this text.
3. Radioimmunoassay [36,40,41] and enzyme immunoassay [42,43] were acceptable as methods for

pepsinogen test definition, as long as results were expressed in nanograms per milliliter (ng/ml) or the equivalent. A pepsinogen test was defined as the measurement of at least pepsinogen I, but ideally of both pepsinogens, and thus the measurment of the pepsinogen I/II ratio. All cutoffs for positivity were considered as long as they were clearly defined or easily determined from the "methods" or "results" sections of the study.

Studies that were not related to the clinical use of pepsinogen for the diagnosis of gastric cancer and which did not contain any data on pepsinogen levels and their variation in relation to gastric lesions were excluded.

A standardized data extraction form was used, after a short period of pilot use by two reviewers. Agreement was obtained on data and studies to be included, and on those data to exclude or not to consider for statistical analysis.

Further assessment of heterogeneity was estimated by using a chi-square test with Meta-DiSc for Windows (version 1.0.9; XI Cochrane Colloquium, Barcelona, Spain). A random-effect model was used for pooling sensitivity, specificity, and the estimated diagnostic odds ratio (DOR), by addressing both within-study sampling error and variation between studies. We assumed an implicit cutoff effect; thus, we considered diagnosis (cancer or precancerous) and the best cutoff after pooling for each outcome.

The time range of the pepsinogen search was from 1982 to 2002.

Forty-two data sets [4,5,11,12,18,44–81] were included: 27 (64%) population-based screening studies ($n = 296553$) and 15 (36%) selected groups ($n = 4385$). Measurement of serum pepsinogen concentration was carried out using either radioimmunoassay [40,41] or enzyme immunoassay [30,42,43]. Homogeneous sensitivity and DOR estimates were found in studies using both pepsinogen I levels and pepsinogen I/II ratio calculations. Pooled pairs of sensitivity and false-positive rates (FPr) for pepsinogen I ≤ 70 ng/ml; pepsinogen I/II ratio ≤ 3, pepsinogen I ≤ 50 ng/ml; pepsinogen I/II ratio ≤ 3, and pepsinogen I ≤ 30 ng/ml; pepsinogen I/II ratio ≤ 2, had sensitivities of 77%/FPr27%, 68%/FPr31%, and 52%/FPr16%, respectively. The positive predictive value (PPV) varied between 0.77% and 1.25%, and the negative predictive value (NPV) varied between 99.08% and 99.90%. In selected groups, pooling was possible only when considering pepsinogen I ≤ 70 ng/ml; pepsinogen I/II ratio ≤ 3: giving sensitivity, 57%; specificity, 80%; PPV, 15%; and NPV, 83%. As for the diagnosis of dysplasia, studies considering pepsinogen I ≤ 50 ng/ml; pepsinogen I/II ratio ≤ 3 obtained a sensitivity of 65% and specificities ranging from 74% to 85%, both with NPV > 95%. We concluded that the definition of the pepsinogen test should include the pepsinogen I/II ratio, as consistency was obtained with this ratio, both in population-based studies and in selected groups for those studies that used pepsinogen I serum levels together with the pepsinogen I/II ratio for screening for gastric cancer in high-incidence regions other than Japan. Further studies of this test in the management of high-risk patients seem to be warranted [39].

A diagnostic test should be reproducible and valid; those tests with a screening purpose, in particular, should be free of discomfort or risk. For the gastrointestinal tract, direct visualization through endoscopic examination is probably the best method for the diagnosis of most protruding and depressed cancer lesions. It easily allows the collection of mucosal specimens for histopathological evaluation, although very high interobserver variability and sampling errors exist for flat lesions and changes, including gastric atrophy, intestinal metaplasia, and even dysplasia. But endoscopic examination is invasive, not patient-friendly, and is not always easily accessible. Furthermore, screeening tests should be economical. Ohata, based on the results of the 2001 annual report of gastric cancer screening prepared by the Japanese Association of Gastrointestinal Mass Screening in 2002, reported that initial screening with conventional barium X-ray cost 3500 yen per subject, and endoscopy cost 13000 yen per subject. The total cost for the screening program was estimated as 25393209000 yen per year. The cost required to find a single case of gastric cancer can be estimated as 4408543 yen, whereas, using the pepsinogen test alone, the cost decreased to 2275387 yen [13]. Therefore, the selection of individuals for endoscopic examination according to the results of noninvasive tests (for instance, the use of the fecal occult blood test [FOBT] for colon and rectum neoplasias) seems to be attractive for most screening programs.

**When should pepsinogens be measured?**

Pepsinogen levels in blood seem to be related to functional changes in the stomach, and their use as serological biopsy has been reported for over 20 years [17,18,71,74]. We focused mainly on the diagnosis of atrophy, as its relation with gastric cancer has been reported. In most Western countries, the focus was on the identification of individuals for intervention studies, whereas in Japan the use of pepsinogen levels was meant to identify those for endoscopic examination, and those at risk for gastric cancer. It is not surprising that studies with different purposes tend to use different methodologies.

Some questions remain unanswered; namely, the consistency of the pepsinogen test in several countries and

population sets, and the definition of the optimal cutoff. It is always ambitious to consider a metaanalysis, because even if all articles are tracked, publication bias is always troublesome. Furthermore, with the previously stated heterogeneity of methods, it is almost an impossible task, and probably for that reason no metaanalysis has been performed before now on the validity of the pepsinogen test for identifying gastric cancer or premalignant gastric lesions. We decided to evaluate the results of several studies and reports, focusing our search mainly on reports from different countries and reports with different purposes (screening or follow-up). We considered addressing the reproducibility of the pepsinogen test by using sensitivity and specificity measures, as these measures show little variation with the prevalence of the disease. Assuming that cutoff points have an intrinsic effect on test validity, we first aimed to assess consistency according to the different cutoff levels used, and then we aimed to pool and define the best discriminatory value for the diagnosis of cancer or other lesions, if possible.

**Factors affecting pepsinogen levels**

Globally, low PPVs were found in population studies. To overcome this problem, some authors tried to adjust cutoff or modify strategies [55,74,75] by measuring confounding factors known to influence pepsinogen levels in blood. From our analysis we were only able to find out that the pepsinogen I/II ratio tended to decrease with age and with the presence of *H. pylori*, but it was not possible to define any modification on cutoff. There were no conclusions in relation to other factors, such as sex or smoking and drinking habits. Age seems to be related to an increase in acid secretion in humans [76]; however, the decrease in pepsinogen I level and the pepsinogen I/II ratio found in most studies may be related not to age but to atrophic changes diagnosed from these findings. The presence of *H. pylori*, assessed either by serological evaluation or by immunohistochemistry in biopsy specimens, in conjunction with inflammation, seems to increase pepsinogen I and II levels and to decrease the pepsinogen I/II ratio [77–79]. Furthermore, as IgG may persist for several years after the disappearance of *H. pylori* infection, its measurement in high-incidence countries may not be effective for diagnosis, as no information is gained. Some authors consider that the value for *H. pylori* negativity is more important. That is, in high-prevalence countries, it may be more important to diagnose an individual with gastric atrophy or other changes as negative for *H. pylori*; this finding could mean that a long time had passed since infection and that mucosal changes had occurred, thus representing a great risk of cancer [13,82–84].

According to our review, around 600 individuals should be screened, using the pepsinogen test, to diagnose one gastric cancer in Japan [13,85,86]. Considering that the main drawback is the positive rate (around 20%), this strategy has to be available at a low price, as it is in Japan (at present, the cost of measuring both pepsinogen I and II can be covered by 1000 yen, which is less than US $10 at current exchange rates). It could be an attractive strategy, as 75% of all gastric cancers discovered in these studies [13,85,86] were early gastric cancers (EGCs) [6,11,80,85], of curable forms with almost 100% 5-year survival. It was possible to evaluate the best strategy for screening as the use of pepsinogen I < 70 ng/ml; pepsinogen I/II ratio < 3. Pooled sensitivity for these values [13,85,86] was 77.3% and specificity, 73.2%. Studies using only pepsinogen I obtained heterogeneous results, even considering obvious differences after cutoff, probably related to other factors, as discussed above. Only the pepsinogen I < 30 ng/ml; pepsinogen I/II ratio < 2 criteria, and not the pepsinogen I < 50 ng/ml; pepsinogen I/II ratio < 3 showed a significant increase in specificity (84%) compared to the pepsinogen I < 70 ng/ml; pepsinogen I/II ratio < 3 criteria.

We also noted very high NPVs in all studies, which did not differ between the population-based studies and the selected group studies (99.9% and 80%, respectively), even considering expected differences in prevalence. This could be the rationale for using the pepsinogen test under follow-up scenarios. As stated above, endoscopy shows low inter-observer agreement as far as neoplastic and non-neoplastic flat lesions are concerned. The use of a noninvasive test; simultaneous measurements of pepsinogen I and II, which reflect all variations in gastric mucosal status, may be able to allow the allocation of some patients, who would otherwise undergo several, eventually inefficacious examinations, to a less intensive follow-up scheme. Screening in Japan has already used this strategy, as a further pepsinogen assay is proposed only 5 years after a negative result in any individual [29,86]. Although no study has specifically analyzed the relationship between the decline of the pepsinogen I/II ratio and the risk of gastric cancer [81], it was noted that variations in the pepsinogen I/II ratio were thought to reflect mainly the advance of atrophy. Other authors [71] showed that a mean pepsinogen I/II ratio of gastric cancer was lower than that for CAG, for dysplasia, and for intestinal metaplasia. In Western countries, where gastric cancer has been declining, these results may be more attractive for early diagnosis strategies, focusing on the follow-up of patients with precancerous lesions. Unfortunately, we were not able to assess and define the best cutoff for this purpose; this inability to define the best cutoff may be related to various factors: the low inter-observer agreement in the endoscopic assessment of atrophy, the biopsy protocol

and sample error, and even differences among pathologists. We can speculate that the cutoff should be the same as the one used for the diagnosis of gastric cancer, as, in fact, the intention is to measure functional changes after atrophy. The highest specificity and NPV were noted when the cutoff pepsinogen I < 30 ng/ml; pepsinogen I/II ratio < 2 was used. As most follow-up programs may be endoscopy-based, the most important factor is to accurately diagnose the absence of disease or severe lesions.

To conclude this section, the use of the same cutoff for positivity of the pepsinogen test obtained similar and comparable results in different sets of individuals and in different countries, both for the diagnosis of such neoplastic gastric lesions as dysplasia and for the diagnosis of carcinoma, a finding that attests to the consistency of the test. Thus, if the pepsinogen test could be made available at a reasonable cost for a screening scenario in high-incidence regions other than in Japan, and for the management of high-risk patients, studies to assess the efficacy and the validity of the test would seem to be worthwhile, as no other noninvasive test has revealed better results to date.

The definition of the pepsinogen test should include the pepsinogen I/II ratio, as homogeneity was obtained both in population-based studies and in selected groups for those studies that used pepsinogen I serum levels together with the pepsinogen I/II ratio. For gastric cancer screening in high-incidence regions other than Japan, and for the management of high-risk patients, further studies using this test would seem to be worthwhile, as stated before.

### Usefulness of gastric cancer screening using the serum pepsinogen test method

To compare the accuracy of the two screening methods — X-ray and pepsinogen test — and to elucidate the usefulness of the serum pepsinogen test method, we performed a study in Toyama Prefecture, which is located in the northern part of Japan [7]. Its total population is about 1 000 000, with a registered gastric cancer death rate, in 1998, of 70.7/100 000 in men and 39.3/100 000 in women. These figures are higher than the mean gastric cancer death rates for the whole country in 1998 (men, 53.6/100 000; women, 27.6/100 000).

This study was specifically designed for the screening of a high-risk gastric cancer group, using the both the X-ray and pepsinogen test methods simultaneously in the same study subjects. They had lived in the same district during the study period, and the incidence (i.e., sensitivity) of detected gastric cancer cases and the PPV were comparable for the two methods, using endoscopy as a yardstick. These results suggest to us that the pepsinogen test method is superior to the conventional X-ray method, although the results of the former may have overestimated the detection of gastric cancer compared with the latter, because the pepsinogen test method was conducted as prevalent screening while the X-ray method was done as incidental screening [87]. To date, few studies have directly examined whether the pepsinogen test method reduced gastric cancer mortality, except for a study in Adachi City in Tokyo and Kake City in Hiroshima Prefecture of Japan [88].

The single use of the pepsinogen test is by no means sufficient for gastric cancer screening; however, it provides a valuable measure for selecting the population that needs further screening with endoscopy [4,18]. As described above, the serum pepsinogen test was introduced for cancer screening to identify individuals with extensive atrophic gastritis [5]. Individuals testing positive for extensive atrophic gastritis by serum pepsinogen levels (pepsinogen I ≤ 70 ng/ml, pepsinogen I/II ratio ≤ 3.0) undergo endoscopic examination to test for the presence of gastric cancer. These test cutoff values have shown a sensitivity of 80%, specificity of 70%, cancer detection rate of 0.44%, and a PPV of 1.5%, using endoscopy as a yardstick [86]. In the past 10 years, a considerable number of screening services provided by workplaces and also by community health services have adopted the pepsinogen serum tests as a primary screening tool [86]. The results of these screenings demonstrate that the cancer detection rate of the screening with the serum tests is superior to and more cost-effective [5–7,12,13,86] than the conventional barium X-ray mass screening. Furthermore, the percentage of early cancers detected by the new serum test screening is higher than that detected by conventional screening, and a considerable number of patients with these early cancers have been successfully treated by endoscopic surgery [11,12,85]. Because the tests detect extensive atrophic gastritis coexisting with cancer, it is possible that the diffuse (poorly differentiated) type [20] of cancer would not be detected by the serum tests. The results of the mass screenings, however, clearly indicate that this is not true, although the serum test screening is especially useful in detecting small asymptomatic cancers, nonulcerated morphology type, and well-differentiated histology type [6,7,11,45,86]. Small asymptomatic cancers of these types are relatively difficult to detect using barium X-rays, whereas conventional screening is good for detecting cancers with an ulcerated morphology type and those with a poorly differentiated histology type, as well as advanced cases, which are frequently symptomatic. Because the cancers detected by the two screening methods are different, the combination of the two screening methods has greatly improved the screening efficacy and is more cost-effective than either method alone [6,7,13,45,86].

Recently, Ohata et al [13] showed a small overlap between the cancers detected by the pepsinogen test and those detected by X-ray screening. We have already recommended several strategies, including concurrent and serial combinations of serum pepsinogen measurement and photofluorography, as well as the single pepsinogen test method [6,7,13,45,86].

In any particular mass screening area, we have to select the best screening system, depending on each individual case, according to the prevalence of gastric cancer, especially in the early stages [45,86]. Although gastric cancer cells are found to produce pepsinogen II more often than pepsinogen I [14–16], elevated pepsinogen values in serum are extremely rare, and only one case has ever been reported in such patients [89], because the amount of pepsinogens which are produced by gastric cancer cells is too small compared to the amount of pepsinogen I and II which are normally secreted into gastric lumen and only 1% of the amount secreted enters the circulation.

## Advantages of the pepsinogen test method

The pepsinogen test method has many advantages compared to the X-ray method. That is, it is more sensitive. It is easy to carry out and patients do not feel much discomfort. There is no radiation exposure, and there are no side effects experienced from barium ingestion. This method is less expensive, it is fast, and many serum samples can be analyzed simultaneously [6,9,12].

## Pepsinogen test kits now available in Japan

Fifteen kinds of pepsinogen test kits, launched by 12 companies, are now available in Japan (Table 1). The pepsinogen test kits are convenient to use and can be used by ancillary medical staff for the measurement of human serum samples, as well as for urine, ascites, and tissue extracts. Therefore, it seems to us that the pepsinogen test method has a promising future.

## Validity of *Helicobacter pylori* antibody titer for gastric cancer screening

In Japan, *H. pylori* infection and other unknown exposure factors may have played an important role in the development of chronic atrophic gastritis [24]. In Japan, *H. pylori* infection is associated with a significantly increased risk of atrophic gastritis [90,91] and the development of gastric cancer, especially early gastric cancer, by providing a suitable environment for carcinogenesis of the gastric mucosa, such as gastric atrophy and intestinal metaplasia [36,92,93]. Extensive atrophy may cause a loss of *H. pylori* infection, with a consequent reduction in the antibody titer. In addition, in advanced gastric cancer, lower antibody titers may be partly attributable to a diminished immune response [82].

## Gastric cancer screening strategy in the near future

In the near future, we should increase the efficacy and cost-effectiveness of gastric cancer screening systems, by the identification of populations at low risk [7,13,83,84,88], as well as those at high risk, of developing gastric cancer. For this purpose, combination assays of the serum *H. pylori* antibody titer and the concentrations of pepsinogen I and II should be used. Both serum measurement and *H. pylori* / CagA assays [46,68,88,94–96] may be beneficial in serological screening strategies,

**Table 1.** List of manufacturers of pepsinogen test kits available in Japan

| Manufacturer | Assay System | Year launched |
|---|---|---|
| Dainabot Co., Ltd. (Abbott Japan Co., Ltd. Tokyo) | IRMA, CLIA | 1992, 2000 |
| Wako Pure Chemical Industries, Ltd. Osaka | EIA, CLEIA | 1997, 2000 |
| Eiken Chemical Co., Ltd. Tokyo | EIA, CLEIA | 1997, 2000 |
| International Reagents Corporation. Kobe | EIA | 1997 |
| Sanwa Kagaku Kenkyusho Co., Ltd. Nagoya | LIA | 1999 |
| Kainos Laboratories, Inc. Tokyo | EIA | 1999 |
| Azwell Inc. Osaka | ELISA | 2000 |
| Kyokuto Pharmaceutical Industrial Co., Ltd. Tokyo | ELISA | 2000 |
| Iatron Laboratories, Inc. (Mitsubishi Kagaku Iatron, Inc. Tokyo) | LIA | 2000 |
| Kyowa Medex Co., Ltd. Tokyo | ELISA | 2001 |
| Shima Laboratories Co., Ltd. Tokyo | LIA | 2002 |
| Fujirebio Inc. Tokyo | EIA | 2005 |

IRMA, immunoradiometric assay; CLIA, chemiluminescent immunoassay; EIA, enzyme immunoassay; CLEIA, chemiluminescent enzyme immunoassay; LIA, latex immunoassay; ELISA, enzyme-linked immunoassay

but cohort studies, evaluating these tests for screening purposes, need to be done.

## Conclusion

In conclusion, the pepsinogen test method can be used as a screening test for high-risk subjects with atrophic gastritis, rather than as a tool for screening for cancer itself. Systematic endoscopic surveillance of this high-risk group is also useful. These strategies would require empirical assessment, using mortality as an endpoint. The international collaboration of health professionals should be encouraged to further advance the prevention and control of this global epidemic. We hope that the new serum pepsinogen test method will become a world standard for gastric cancer prevention in the near future. We also hope that, in other countries (especially in developing countries, which have high incidences of gastric cancer), as well as in Japan, there will be improvements in endoscopic skills in diagnosing early gastric cancers with subtle mucosal changes.

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